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File: USPT

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DOCUMENT-IDENTIFIER: US 4767701 A

TITLE: Poly I:C covalently bonded to polymer for diagnostic purposes

Brief Summary Text (22):

Immobilisation of biologically active substances to carboxyl functional carriers is previously known in and per se and is used, inter alia, for enzymes and other proteins. Covalent immobilisation of double-stranded polynucleotides, such as Poly I:C, on the other hand is complicated and, as far as is known, has not been described in literature. All known immobilisation techniques aiming at providing covalent bonds require, in the biologically active substance, some functional group which either directly or via a linkage can be made to couple to the functional group of the carrier material. As mentioned above, Poly I:C has amino groups in purine and pyrimidine bases which in and per se could be made to react with carboxyl groups in the surface of the carrier polymer but which, in actual practice, are entirely inert because they are utilised in hydrogen bonds between the chains.

Brief Summary Text (27):

The reaction mechanism of this immobilisation technique has not been explained, but one theory is that the two polynucleotide strands upon heating at least partly separate into individual polymer chains. The water-soluble carbodiimide then makes it possible to form amide bonds between primary amino groups in the cytosine of the poly C chains and carboxyl groups on the carrier surface. The double-stranded structure is then at least partly reformed upon cooling, i.e. the poly I chains are withdrawn from the solution and again bonded by means of hydrogen bonds to the immobilised complementary strand. As will appear from the following Examples, the biological effect of the immobilised polynucleotide is excellent, which should imply that the original conformation of the double strand has largely been reformed.